

TREATMENT OF CARDIOVASCULAR AND RELATED PATHOLOGIES

[0001] This application claims priority to provisional application Serial No. 60/150,415 filed on August 24, 1999. This application is a divisional of application Serial No. 09/645,237 filed on August 24, 2000.

FIELD OF THE INVENTION

[0002] This invention relates to methods of treating cardiovascular and related diseases, such as hypertrophy, hypertension, congestive heart failure, ischemia, such as myocardial ischemia, ischemia reperfusion injuries in various organs, arrhythmia, and myocardial infarction.

BACKGROUND

[0003] Heart failure is a pathophysiological condition in which the heart is unable to pump blood at a rate commensurate with the requirement of the metabolizing tissues or can do so only from an elevated filling pressure (increased load). Thus, the heart has a diminished ability to keep up with its workload. Over time, this condition leads to excess fluid accumulation, such as peripheral edema, and is referred to as congestive heart failure.

[0004] When an excessive pressure or volume load is imposed on a ventricle, myocardial hypertrophy (i.e., enlargement of the heart muscle) develops as a compensatory mechanism. Hypertrophy permits the ventricle to sustain an increased load because the heart muscle can contract with greater force. However, a ventricle subjected to an abnormally elevated load for a prolonged period eventually fails to sustain an increased load despite the presence of ventricular hypertrophy, and pump failure may ultimately occur.

[0005] Heart failure can arise from any disease that affects the heart and interferes with circulation. For example, a disease that increases the heart muscle's workload, such as hypertension, will eventually weaken the force of the heart's contraction. Hypertension is a condition in which there is an increase in resistance to blood flow through the vascular system. This resistance leads to increases in systolic and/or diastolic blood pressures. Hypertension places increased tension to the left ventricular myocardium, causing it to stiffen and hypertrophy,

and accelerates the development of atherosclerosis in the coronary arteries. The combination of increased demand and lessened supply increases the likelihood of myocardial ischemia leading to myocardial infarction, sudden death, arrhythmias, and congestive heart failure.

[0006] Ischemia is a condition in which an organ or a part of the body fails to receive a sufficient blood supply. When an organ is deprived of its blood supply, it is said to be hypoxic. An organ will become hypoxic even when the blood supply temporarily ceases, such as during a surgical procedure or during temporary artery blockage. Ischemia initially leads to a decrease in or loss of contractile activity. When the organ affected is the heart, this condition is known as myocardial ischemia, and myocardial ischemia initially leads to abnormal electrical activity. This may generate an arrhythmia. When myocardial ischemia is of sufficient severity and duration, cell injury may progress to cell death—i.e., myocardial infarction—and subsequently to heart failure, hypertrophy, or congestive heart failure.

[0007] When blood flow resumes to an organ after temporary cessation, this is known as ischemic reperfusion of the organ. For example, reperfusion of an ischemic myocardium may counter the effects of coronary occlusion, a condition that leads to myocardial ischemia. Ischemic reperfusion to the myocardium may lead to reperfusion arrhythmia or reperfusion injury. The severity of reperfusion injury is affected by numerous factors, such as, for example, duration of ischemia, severity of ischemia, and speed of reperfusion. Conditions observed with ischemia reperfusion injury include neutrophil infiltration, necrosis, and apoptosis.

[0008] Drug therapies, using known active ingredients such as vasodilators, angiotensin II receptor antagonists, angiotensin converting enzyme inhibitors, diuretics, antithrombolytic agents, β -adrenergic receptor antagonists, α -adrenergic receptor antagonists, calcium channel blockers, and the like, are available for treating heart failure and associated diseases. Of course, any drug used for treatment may result in side effects. For example, vasodilators may result in hypotension, myocardial infarction, and adverse immune response. Angiotensin II receptor antagonists and angiotensin converting enzyme inhibitors are often associated with acute renal failure, fetopathic potential, proteinuria, hepatotoxicity, and glycosuria as side effects. Similarly, common side effects associated with calcium channel blockers include hypotension, peripheral

edema, and pulmonary edema. β -Adrenergic receptor antagonists and diuretics have been associated with incompatibility with nonsteroidal anti-inflammatory drugs in addition to impotence, gout, and muscle cramps in the case of diuretics and in addition to a decrease in left ventricular function and sudden withdrawal syndrome in the case of β -adrenergic receptor antagonists. Moreover, side effects associated with α -adrenergic receptor antagonists include thostatic hypotension, and side effects associated with antithrombolytic agents include excessive bleeding.

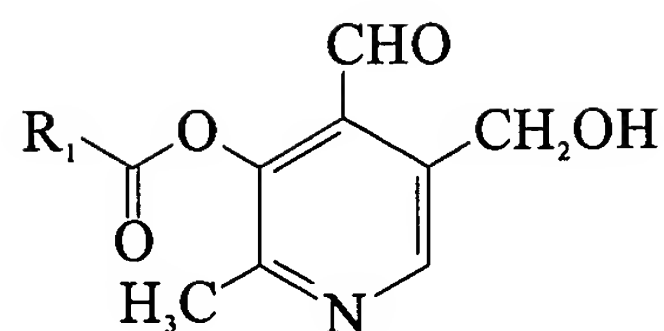
[0009] To address the side effects, the dosage of a drug may be reduced or the administration of the drug may be abated and replaced with another drug. It would be desirable to administer a drug therapy with decreased amounts of the active ingredient to reduce side effects but maintain effectiveness.

SUMMARY OF THE INVENTION

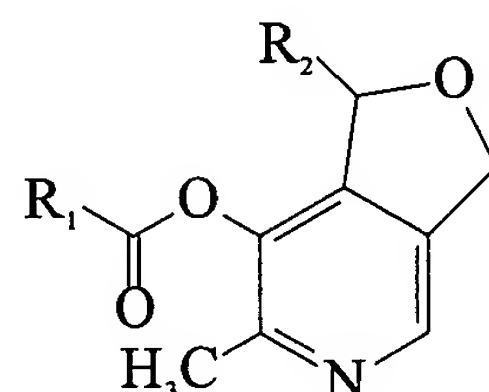
[0010] The present invention provides methods for treating cardiovascular and related diseases, such as, for example, hypertrophy, hypertension, congestive heart failure, myocardial ischemia, ischemia reperfusion injuries in an organ, arrhythmia, and myocardial infarction. One embodiment is directed to a method of treating cardiovascular disease in a mammal by concurrently administering to the mammal a therapeutically effective amount of a combination of a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound. Therapeutic cardiovascular compounds suitable for use in methods of the invention include an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, an antithrombolytic agent, a β -adrenergic receptor antagonist, a vasodilator, a diuretic, an α -adrenergic receptor antagonist, an antioxidant, and a mixture thereof. In some embodiments, the therapeutic cardiovascular compound is PPADS.

[0011] Compounds suitable for use in the methods of the invention include pyridoxal-5'-phosphate, pyridoxamine, pyridoxal, 3-acylated pyridoxal analogues, pharmaceutically acceptable acid addition salts thereof, and mixtures thereof.

[0012] In one embodiment, a 3-acylated pyridoxal analogue is a compound of the formula



[0013] In another embodiment, a 3-acylated pyridoxal analogue is a compound of the formula



BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on mortality in the rat model of coronary ligation.

[0015] Figure 2 is a graph showing the effect of P-5-P and captopril, alone or in combination, on mortality in the rat model of coronary ligation.

[0016] Figure 3 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on mortality in the rat model of coronary ligation.

[0017] Figure 4 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on mortality in the rat model of coronary ligation.

[0018] Figure 5 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on scar weight in the rat model of coronary ligation.

[0019] Figure 6 is a graph showing the effect of P-5-P and captopril, alone or in combination, on scar weight in the rat model of coronary ligation.

[0020] Figure 7 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on scar weight in the rat model of coronary ligation.

[0021] Figure 8 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on scar weight in the rat model of coronary ligation.

- [0022]** Figure 9 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on the rate of force of contraction ($+dp/dt$) in the rat model of coronary ligation.
- [0023]** Figure 10 is a graph showing the effect of P-5-P and captopril, alone or in combination, on the rate of force of contraction ($+dp/dt$) in the rat model of coronary ligation.
- [0024]** Figure 11 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on the rate of force of contraction ($+dp/dt$) in the rat model of coronary ligation.
- [0025]** Figure 12 is a graph showing the effect of P-5-P verapamil, alone or in combination, on the rate of force of contraction ($+dp/dt$) in the rat model of coronary ligation.
- [0026]** Figure 13 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on the rate of force of relaxation ($-dp/dt$) in the rat model of coronary ligation.
- [0027]** Figure 14 is a graph showing the effect of P-5-P and captopril, alone or in combination, on the rate of force of relaxation ($-dp/dt$) in the rat model of coronary ligation.
- [0028]** Figure 15 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on the rate of force of relaxation ($-dp/dt$) in the rat model of coronary ligation.
- [0029]** Figure 16 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on the rate of force of relaxation ($-dp/dt$) in the rat model of coronary ligation.
- [0030]** Figure 17 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on left ventricular end diastolic pressure (LVEDP) in the rat model of coronary ligation.
- [0031]** Figure 18 is a graph showing the effect of P-5-P and captopril, alone or in combination, on left ventricular end diastolic pressure (LVEDP) in the rat model of coronary ligation.
- [0032]** Figure 19 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on left ventricular end diastolic pressure (LVEDP) in the rat model of coronary ligation.
- [0033]** Figure 20 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on left ventricular end diastolic pressure (LVEDP) in the rat model of coronary ligation.
- [0034]** Figure 21 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on heart weight in the rat model of coronary ligation.
- [0035]** Figure 22 is a graph showing the effect of P-5-P and captopril, alone or in combination, on heart weight in the rat model of coronary ligation.

[0036] Figure 23 is a graph showing the effect of P-5-P propranolol, alone or in combination, on heart weight in the rat model of coronary ligation.

[0037] Figure 24 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on heart weight in the rat model of coronary ligation.

[0038] Figure 25 is a graph showing the effect of P-5-P and aspirin, alone or in combination, on right ventricular weight in the rat model of coronary ligation.

[0039] Figure 26 is a graph showing the effect of P-5-P and captopril, alone or in combination, on right ventricular weight in the rat model of coronary ligation.

[0040] Figure 27 is a graph showing the effect of P-5-P and propranolol, alone or in combination, on right ventricular weight in the rat model of coronary ligation.

[0041] Figure 28 is a graph showing the effect of P-5-P and verapamil, alone or in combination, on right ventricular weight in the rat model of coronary ligation.

[0042] Figure 29A is a graph showing systolic blood pressure in rats from all pretreatment experiment groups at "0" day. "C" designates a control group; "S" designates a sucrose diet induced diabetic group; "M" designates a group administered P-5-P alone; "Ca" designates a group administered captopril alone; "V" designates a group administered verapamil alone; "M+Ca" designates a group administered P-5-P and captopril; "M+V" designates a group administered P-5-P and verapamil.

[0043] Figure 29B is a graph showing the effect of pretreatment with P-5-P, captopril and verapamil on systolic blood pressure in rats when administered 1 week prior to sucrose diet induced diabetes. "C", "S", "M", "Ca", "V", "M+Ca", and "M+V" are designated as in Figure 29A.

[0044] Figure 30A is a graph showing systolic blood pressure in rats from all experiment groups involved in same day treatment as sucrose feeding at "0" day. "C", "S", "M", "Ca", "V", "M+Ca", and "M+V" are designated as in Figure 29A.

[0045] Figure 30B is a graph showing the effect of administration of P-5-P, captopril and verapamil on systolic blood pressure in rats when administered the same day as sucrose feeding to induce diabetes. "C", "S", "M", "Ca", "V", "M+Ca", and "M+V" are designated as in Figure 29A.

[0046] Figure 31A is a graph showing systolic blood pressure in rats from all experiment groups involved in treatment two weeks after sucrose feeding at "0" day. "C", "S", "M", "Ca", "V", "M+Ca", and "M+V" are designated as in Figure 29A.

[0047] Figure 31B is a showing systolic blood pressure in rats from all experiment groups involved in treatment two weeks after sucrose feeding at "0" day. "C", "S", "M", "Ca", "V", "M+Ca", and "M+V" are designated as in Figure 29A.

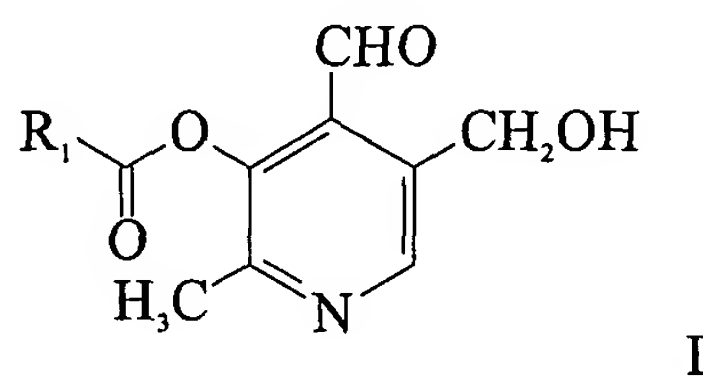
DESCRIPTION OF THE INVENTION

[0048] The present invention provides methods for treatment of cardiovascular and related diseases or conditions. Such cardiovascular and related diseases include hypertrophy, hypertension, congestive heart failure, ischemia, such as myocardial ischemia, ischemia reperfusion injury, arrhythmia, and myocardial infarction.

[0049] In accordance with the present invention, it has been found that pyridoxal-5'-phosphate and its derivatives can be used concurrently with therapeutic cardiovascular compounds in the treatment of the above-identified diseases and conditions. "Treatment" and "treating" as used herein include preventing, inhibiting, and alleviating cardiovascular diseases, related diseases, and related symptoms as well as healing the ischemia-related conditions or symptoms thereof affecting mammalian organs and tissues. Treatment may be carried out by concurrently administering a therapeutically effective amount of a combination of a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound.

[0050] A "therapeutically effective amount" as used herein includes a prophylactic amount, for example, an amount effective for preventing or protecting against cardiovascular diseases, related diseases, and symptoms thereof, and amounts effective for alleviating or healing cardiovascular diseases, related diseases, and symptoms thereof. By administering a compound suitable for use in methods of the invention concurrently with a therapeutic cardiovascular compound, the therapeutic cardiovascular compound may be administered in a dosage amount that is less than the dosage amount required when the therapeutic cardiovascular compound is administered as a sole active ingredient. By administering lower dosage amounts of the active ingredient, the side effects associated therewith should accordingly be reduced.

[0051] Compounds suitable for use in the methods of the invention include pyridoxal-5'-phosphate, pyridoxal, pyridoxamine, 3-acylated pyridoxal analogues, pharmaceutically acceptable acid addition salts thereof, and mixtures thereof. 3-Acylated pyridoxal analogues provide for slower metabolism to pyridoxal *in vivo*. For example, a suitable 3-acylated analogue of pyridoxal (2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine) is a compound of the formula I:



or a pharmaceutically acceptable acid addition salt thereof, wherein

[0052] R_1 is a straight or branched alkyl group, a straight or branched alkenyl group, in which an alkyl or alkenyl group may be interrupted by a nitrogen or oxygen atom; an alkoxy group; a dialkylamino group; or an unsubstituted or substituted aryl group.

[0053] The term "alkyl" group includes a straight or branched saturated aliphatic hydrocarbon chain having from 1 to 8 carbon atoms, such as, for example, methyl, ethyl, propyl, isopropyl (1-methylethyl), butyl, *tert*-butyl (1,1-dimethylethyl), and the like.

[0054] The term "alkenyl" group includes an unsaturated aliphatic hydrocarbon chain having from 2 to 8 carbon atoms, such as, for example, ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-methyl-1-propenyl, and the like.

[0055] The above alkyl or alkenyl groups may optionally be interrupted in the chain by a heteroatom, such as, for example, a nitrogen or oxygen atom, forming an alkylaminoalkyl or alkoxyalkyl group, for example, methylaminoethyl or methoxymethyl, and the like.

[0056] The term "alkoxy" group includes an alkyl group as defined above joined to an oxygen atom having preferably from 1 to 4 carbon atoms in a straight or branched chain, such as, for

example, methoxy, ethoxy, propoxy, isopropoxy (1-methylethoxy), butoxy, *tert*-butoxy (1,1-dimethylethoxy), and the like.

[0057] The term "dialkylamino" group includes two alkyl groups as defined above joined to a nitrogen atom, in which the alkyl group has preferably 1 to 4 carbon atoms, such as, for example, dimethylamino, diethylamino, methylethylamino, methylpropylamino, diethylamino, and the like.

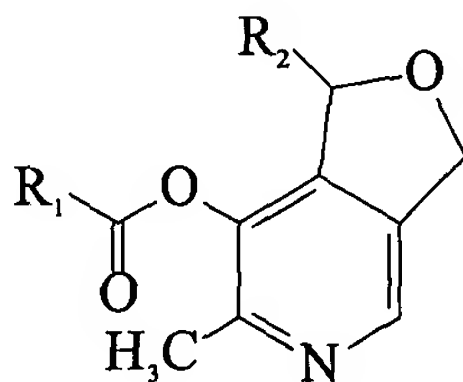
[0058] The term "aryl" group includes an aromatic hydrocarbon group, including fused aromatic rings, such as, for example, phenyl and naphthyl. Such groups may be unsubstituted or substituted on the aromatic ring by, for example, an alkyl group of 1 to 4 carbon atoms, an alkoxy group of 1 to 4 carbon atoms, an amino group, a hydroxy group, or an acetyloxy group.

[0059] Preferred R₁ groups for compounds of formula I are toluyl or naphthyl. Such

R₁ groups when joined with a carbonyl group form an acyl group $\text{R}_1\text{C}(=\text{O})-$ which preferred for compounds of formula I include toluoyl or β -naphthoyl. Of the toluoyl group, the *p*-isomer is more preferred.

[0060] Examples of 3-acylated analogues of pyridoxal include, but are not limited to, 2-methyl-3-toluoyloxy-4-formyl-5-hydroxymethylpyridine and 2-methyl- β -naphthoyloxy-4-formyl-5-hydroxymethylpyridine.

[0061] Another suitable analogue is a 3-acylated analogue of pyridoxal-4,5-aminal (1-secondary amino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-*c*)pyridine) of the formula II:



II

or a pharmaceutically acceptable acid addition salt thereof, wherein

[0062] R_1 is a straight or branched alkyl group, a straight or branched alkenyl group, in which an alkyl or alkenyl group may be interrupted by a nitrogen or oxygen atom; an alkoxy group; a dialkylamino group; or an unsubstituted or substituted aryl group; and

[0063] R_2 is a secondary amino group.

[0064] The terms "alkyl," "alkenyl," "alkoxy," "dialkylamino," and "aryl" are as defined above.

[0065] The term "secondary amino" group includes a group of the formula III:



derived from a secondary amine R_3R_4NH , in which R_3 and R_4 are each independently alkyl, alkenyl, cycloalkyl, aryl, or, when R_3 and R_4 are taken together, may form a ring with the nitrogen atom and which may optionally be interrupted by a heteroatom, such as, for example, a nitrogen or oxygen atom. The terms "alkyl," "alkenyl," and "aryl" are used as defined above in forming secondary amino groups such as, for example, dimethylamino, methylethylamino, diethylamino, dialkylamino, phenylmethylamino, diphenylamino, and the like.

[0066] The term "cycloalkyl" refers to a saturated hydrocarbon having from 3 to 8 carbon atoms, preferably 3 to 6 carbon atoms, such as, for example, cyclopropyl, cyclopentyl, cyclohexyl, and the like.

[0067] When R_3 and R_4 are taken together with the nitrogen atom, they may form a cyclic secondary amino group, such as, for example, piperidino, and, when interrupted with a heteroatom, includes, for example, piperazino and morpholino.

[0068] Preferred R₁ groups for compounds of formula II include toluyl, e.g., *p*-toluyl, naphthyl, *tert*-butyl, dimethylamino, acetylphenyl, hydroxyphenyl, or alkoxy, e.g., methoxy.

Such R₁ groups when joined with a carbonyl group form an acyl group $\text{R}_1\overset{\text{O}}{\parallel}\text{C}-$ which preferred for compounds of formula II include toluoyl, β -naphthoyl, pivaloyl, dimethylcarbamoyl, acetylsalicyloyl, salicyloyl, or alkoxycarbonyl. A preferred secondary amino group may be morpholino.

[0069] Examples of 3-acylated analogues of pyridoxal-4,5-aminal include, but are not limited to, 1-morpholino-1,3-dihydro-7-(*p*-toluoyloxy)-6-methylfuro(3,4-*c*)pyridine; 1-morpholino-1,3-dihydro-7-(β -naphthoyloxy)-6-methylfuro(3,4-*c*)pyridine; 1-morpholino-1,3-dihydro-7-pivaloyloxy-6-methylfuro(3,4-*c*)pyridine; 1-morpholino-1,3-dihydro-7-carbamoyloxy-6-methylfuro(3,4-*c*)pyridine; and 1-morpholino-1,3-dihydro-7-acetylsalicyloxy-6-methylfuro(3,4-*c*)pyridine.

[0070] The compounds of formula I may be prepared by reacting pyridoxal hydrochloride

with an acyl halide in an aprotic solvent. A suitable acyl group is $\text{R}_1\overset{\text{O}}{\parallel}\text{C}-$, wherein R₁ is as defined above. A particularly suitable acyl halide includes *p*-toluoyl chloride or β -naphthoyl chloride. A suitable aprotic solvent includes acetone, methylethylketone, and the like.

[0071] The compounds of formula II may be prepared by reacting 1-secondary amino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-*c*)pyridine with an acyl halide in an aprotic solvent. An

acyl group is $\text{R}_1\overset{\text{O}}{\parallel}\text{C}-$, wherein R₁ is as defined above. A particularly suitable acyl halide includes *p*-toluoyl chloride, β -naphthoyl chloride, trimethylacetyl chloride, dimethylcarbamoyl chloride, and acetylsalicyloyl chloride. A particularly suitable secondary amino group includes morpholino.

[0072] The compound 1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-*c*)pyridine may be prepared by methods known in the art, for example, by reacting morpholine and pyridoxal hydrochloride at a temperature of about 100°C in a solvent. A suitable solvent includes, for example, toluene. Similarly, other secondary amines as defined for R₂ may be used as reactants to prepare the appropriate 1-secondary amino compounds.

[0073] The compounds of formula I may alternatively be prepared from the compounds of formula II by reacting a compound of formula II with an aqueous acid, such as, for example, aqueous acetic acid.

[0074] One skilled in the art would recognize variations in the sequence and would recognize variations in the appropriate reaction conditions from the analogous reactions shown or otherwise known that may be appropriately used in the above-described processes to make the compounds of formulas I and II herein.

[0075] The products of the reactions described herein are isolated by conventional means such as extraction, distillation, chromatography, and the like.

[0076] Pharmaceutically acceptable acid addition salts of compounds suitable for use in methods of the invention include salts derived from nontoxic inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, hydrofluoric, phosphorous, and the like, as well as the salts derived from nontoxic organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, trifluoroacetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are salts of amino acids

such as arginate and the like and gluconate, galacturonate, n-methyl glutamine, etc. (see, e.g., Berge et al., *J. Pharmaceutical Science*, 66: 1-19 (1977)).

[0077] The acid addition salts of the basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

[0078] Methods of the invention include concurrently administering pyridoxal-5'-phosphate, pyridoxamine, pyridoxal, a 3-acylated pyridoxal analogue, a pharmaceutically acceptable acid addition salt thereof, or a mixture thereof with a therapeutic cardiovascular compound to treat hypertrophy, hypertension, congestive heart failure, ischemia, such as myocardial ischemia, ischemia reperfusion injury, arrhythmia, or myocardial infarction. Preferably, the cardiovascular disease treated is hypertrophy or congestive heart failure. Still preferably, the cardiovascular disease treated is arrhythmia. Also preferably, the cardiovascular disease treated is ischemia reperfusion injury.

[0079] Therapeutic cardiovascular compounds that may be concurrently administered with a compound suitable for use in methods of the invention include an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, an antithrombolytic agent, a β -adrenergic receptor antagonist, a vasodilator, a diuretic, an α -adrenergic receptor antagonist, an antioxidant, and a mixture thereof. A compound suitable for use in methods of the invention also may be concurrently administered with PPADS (pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid), also a therapeutic cardiovascular compound, or with PPADS and another known therapeutic cardiovascular compound as already described. In a preferred embodiment, pyridoxal-5'-phosphate is concurrently administered with PPADS or with PPADS and another known therapeutic cardiovascular compound, preferably an angiotensin converting enzyme inhibitor or an angiotensin II receptor antagonist.

[0080] Preferably, a therapeutic cardiovascular compound, which is concurrently administered with pyridoxal-5'-phosphate, pyridoxamine, pyridoxal, a 3-acylated pyridoxal analogue, a pharmaceutically acceptable acid addition salt thereof, or a mixture thereof, is an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, or a diuretic. Still preferably, the therapeutic cardiovascular compound is an α -adrenergic receptor antagonist. Also preferably, the therapeutic cardiovascular compound is a calcium channel blocker.

[0081] These therapeutic cardiovascular compounds are generally used to treat cardiovascular and related diseases as well as symptoms thereof. A skilled physician or veterinarian readily determines a subject who is exhibiting symptoms of any one or more of the diseases described above and makes the determination about which compound is generally suitable for treating specific cardiovascular conditions and symptoms.

[0082] For example, myocardial ischemia may be treated by the administration of, for example, angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, an antithrombolytic agent, a β -adrenergic receptor antagonist, a diuretic, an α -adrenergic receptor antagonist, or a mixture thereof. In some instances, congestive heart failure may be treated by the administration of, for example, angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, a vasodilator, a diuretic, or a mixture thereof.

[0083] Myocardial infarction may be treated by the administration of, for example, angiotensin converting enzyme inhibitor, a calcium channel blocker, an antithrombolytic agent, a β -adrenergic receptor antagonist, a diuretic, an α -adrenergic receptor antagonist, or a mixture thereof.

[0084] Hypertension may be treated by the administration of, for example, angiotensin converting enzyme inhibitor, a calcium channel blocker, a β -adrenergic receptor antagonist, a vasodilator, a diuretic, an α -adrenergic receptor antagonist, or a mixture thereof.

[0085] Moreover, arrhythmia may be treated by the administration of, for example, a calcium channel blocker, a β -adrenergic receptor antagonist, or a mixture thereof.

[0086] Antithrombolytic agents are used for reducing or removing blood clots from arteries.

[0087] Hypertrophy may be treated by the administration of, for example, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, or a mixture thereof.

[0088] Ischemia reperfusion injury may be treated by the administration of, for example, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, a calcium channel blocker, or a mixture thereof.

[0089] Known angiotensin converting enzyme inhibitors include, for example, captopril, enalapril, lisinopril, benazapril, fosinopril, quinapril, ramipril, spirapril, imidapril, and moexipril.

[0090] Examples of known angiotensin II receptor antagonists include both angiotensin I receptor subtype antagonists and angiotensin II receptor subtype antagonists. Suitable angiotensin II receptor antagonists include losartan and valsartan.

[0091] Suitable calcium channel blockers include, for example, verapamil, diltiazem, nicardipine, nifedipine, amlodipine, felodipine, nimodipine, and bepridil.

[0092] Antithrombolytic agents known in the art include antiplatelet agents, aspirin, and heparin.

[0093] Examples of known β -adrenergic receptor antagonists include atenolol, propranolol, timolol, and metoprolol.

[0094] Suitable vasodilators include, for example, hydralazine, nitroglycerin, and isosorbide dinitrate.

[0095] Suitable diuretics include, for example, furosemide, diuril, amiloride, and hydrodiuril.

[0096] Suitable α -adrenergic receptor antagonists include, for example, prazosin, doxazocin, and labetalol.

[0097] Suitable antioxidants include vitamin E, vitamin C, and isoflavones.

[0098] A compound suitable for use in methods of the invention and a therapeutic cardiovascular compound are administered concurrently. "Concurrent administration" and "concurrently administering" as used herein includes administering a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound in admixture, such as, for example, in a pharmaceutical composition or in solution, or as separate compounds, such as, for example, separate pharmaceutical compositions or solutions administered consecutively, simultaneously, or at different times but not so distant in time such that the compound suitable for use in methods of the invention and the therapeutic cardiovascular compound cannot interact and a lower dosage amount of the active ingredient cannot be administered.

[0099] A physician or veterinarian of ordinary skill readily determines a subject who is exhibiting symptoms of any one or more of the diseases described above. Regardless of the route of administration selected, the compound suitable for use in methods of the invention and the therapeutic cardiovascular compound are formulated into pharmaceutically acceptable unit dosage forms by conventional methods known to the pharmaceutical art. An effective but nontoxic quantity of the compound suitable for use in methods of the invention and the therapeutic cardiovascular compound are employed in the treatment.

[0100] The compound suitable for use in methods of the invention and the therapeutic cardiovascular compound may be concurrently administered enterally and/or parenterally in admixture or separately. Parenteral administration includes subcutaneous, intramuscular, intradermal, intramammary, intravenous, and other administrative methods known in the art. Enteral administration includes tablets, sustained release tablets, enteric coated tablets, capsules,

sustained release capsules, enteric coated capsules, pills, powders, granules, solutions, and the like.

[0101] A pharmaceutical composition suitable for administration comprises a pharmaceutically acceptable carrier and a compound suitable for use in methods of the invention and/or a therapeutic cardiovascular compound. The pharmaceutical composition comprises a pharmaceutically acceptable carrier and a compound suitable for use in methods of the invention, such as, for example, pyridoxal-5'-phosphate, pyridoxal, pyridoxamine, a 3-acylated pyridoxal analogue, a pharmaceutically acceptable acid addition salt thereof, and a mixture thereof. A pharmaceutically acceptable carrier includes, but is not limited to, physiological saline, ringers, phosphate buffered saline, and other carriers known in the art. Pharmaceutical compositions may also include stabilizers, antioxidants, colorants, and diluents. Pharmaceutically acceptable carriers and additives are chosen such that side effects from the pharmaceutical compound are reduced or minimized and the performance of the compound is not canceled or inhibited to such an extent that treatment is ineffective.

[0102] Methods of preparing pharmaceutical compositions containing a pharmaceutically acceptable carrier and a compound suitable for use in methods of the invention and/or a therapeutic cardiovascular compound are known to those of skill in the art. All methods may include the step of bringing the compound suitable for use in methods of the invention and/or the therapeutic cardiovascular compound in association with the carrier or additives. In general, the formulations are prepared by uniformly and intimately bringing the compound into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired unit dosage form.

[0103] The ordinarily skilled physician or veterinarian will readily determine and prescribe the therapeutically effective amount of the compound to treat the disease for which treatment is administered. In so proceeding, the physician or veterinarian could employ relatively low dosages at first, subsequently increasing the dose until a maximum response is obtained. Typically, the particular disease, the severity of the disease, the compound to be administered, the route of administration, and the characteristics of the mammal to be treated, for example, age,

sex, and weight, are considered in determining the effective amount to administer.

Administering a therapeutically effective amount of a compound suitable for use in methods of the invention to treat cardiovascular and related diseases as well as symptoms thereof is typically in a range of about 0.1-100 mg/kg of a patient's body weight, more preferably in the range of about 0.5-50 mg/kg of a patient's body weight per daily dose when administered alone. The compound suitable for use in methods of the invention may be administered for periods of short and long duration.

[0104] Therapeutically effective amounts of respective therapeutic cardiovascular compounds when administered as sole active ingredients are known in the art and may be found in, for example, Physicians' Desk Reference (53rd ed., 1999).

[0105] When concurrently administering a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound, the compound suitable for use in methods of the invention is typically administered in a range of about 0.1-100 mg/kg of a patient's body weight, preferably 0.5-50 mg/kg of a patient's body weight, per daily dose, and the therapeutic cardiovascular compound is administered in an amount less than the amount known in the art, which is administered when the therapeutic cardiovascular compound is administered as the sole active ingredient. Typically, the therapeutic cardiovascular compound is administered in an amount at least 5% less than the amount known in the art, which is administered when the therapeutic cardiovascular compound is administered as the sole active ingredient.

[0106] A therapeutically effective amount of a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound for treating cardiovascular and related diseases and symptoms thereof can be administered prior to, concurrently with, or after the onset of the disease or symptom. For example, a therapeutically effective amount of a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound for treating ischemia reperfusion injury or myocardial infarction can be administered before, during, or following ischemia (including during or following reperfusion), as well as continually for some period spanning from pre- to post-ischemia. For example, the compound suitable for use in methods of the invention and a therapeutic cardiovascular compound may be concurrently

administered prior to heart procedures, including bypass surgery, thrombolysis, and angioplasty, and prior to any other procedures that require blood flow be interrupted and then resumed. Additionally, a compound suitable for use in methods of the invention and a therapeutic cardiovascular compound may be taken on a regular basis to protect against cellular dysfunction arising from arrhythmia and heart failure.

[0107] The invention is further elaborated by the representative examples as follows. Such examples are not meant to be limiting.

EXAMPLES

Example 1: Synthesis of morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-c)pyridine)

[0108] A mixture of morpholine (20g) and toluene (100mL) was stirred and heated using an oil bath set to 100°C for 15 minutes. Pyridoxal hydrochloride (10g) was then added and the reaction mixture was stirred at 100°C for two hours. The reaction mixture was then concentrated by distillation of the toluene and morpholine. The concentrated reaction mixture was washed three times by adding toluene (100mL) and removing the toluene by distillation. After washing, the residue was dissolved in toluene and filtered, and then hexane was added until precipitation began, at which time the reaction mixture was left overnight at room temperature. Crystals were collected and washed thoroughly with hexane.

[0109] Nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy confirmed the identity of the synthesized compound. The purity of the compound was analyzed by high performance liquid chromatography (HPLC) using a C-18 reverse phase column and water/acetonitrile as solvent (1-100% acetonitrile over 25 minutes).

Example 2: Synthesis of the 3-toluate of the morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-(*p*-toluoyloxy)-6-methylfuro(3,4-c)pyridine)

[0110] Anhydrous powdered potassium carbonate (5g), acetone (100mL), and morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-c)pyridine) (1.11 g, 5 mmoles) were mixed in a nitrogen-cooled, dry flask. The reaction mixture was cooled to

between 0 and 5°C and then *p*-toluoyl chloride (1.06g, 6 mmoles) in acetone (20mL) was added. This mixture was stirred for two hours, followed by filtering out the solid and evaporating the solution to dryness under vacuum. The residue was chromatographed on silica gel using a mixture of ethyl acetate and hexane as solvent.

[0111] The purified solid was analyzed by thin layer chromatography (TLC), NMR, and mass spectroscopy. The purity of the synthesized compound was confirmed by HPLC as described in Example 1.

Example 3: Synthesis of the 3-toluate of pyridoxal (2-methyl-3-toluoyloxy-4-formyl-5-hydroxymethylpyridine)

[0112] Anhydrous potassium carbonate (10g), acetone (100mL), and pyridoxal hydrochloride (2.03g, 10 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then *p*-toluoyl chloride (2.12g, 12 mmoles) in acetone (20mL) was added. The reaction mixture was stirred for two hours followed by filtering out the solid and evaporating the solution to dryness under vacuum. The residue was chromatographed on silica gel as described in Example 2.

[0113] The purified solid was analyzed by TLC, NMR, and mass spectroscopy. The purity of the compound was confirmed by HPLC as described in Example 1.

[0114] Alternative to the above-described method, the 3-toluate of pyridoxal is synthesized by reacting the compound of Example 2 with 80% aqueous acetic acid at 60°C for 30 minutes, and then diluting with water and extracting by ethyl acetate. The ethyl acetate layer is washed with 5% aqueous sodium bicarbonate, dried with magnesium sulfate, and evaporated to dryness. The compound is also analyzed as described *supra*.

Example 4: Synthesis of 3- β -naphthoate of the morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-(β -naphthoyloxy)-6-methylfuro(3,4-*c*)pyridine)

[0115] Anhydrous powdered potassium carbonate (5g), acetone (100mL), and morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-*c*)pyridine)

(1.11g, 5 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then β -naphthoyl chloride (1.06g, 6 mmoles) in acetone (20mL) was added. The reaction mixture was stirred for two hours, and then the solid was filtered out and the solution was evaporated to dryness under vacuum. The residue was chromatographed according to Example 2.

[0116] The purified solid was analyzed according to Example 2, and the purity was confirmed according to Example 1.

Example 5: Synthesis of the 3- β -naphthoate of pyridoxal (2-methyl-3- β -naphthoxyloxy-4-formyl-5-hydroxymethylpyridine)

[0117] Anhydrous potassium carbonate (10g), acetone (100mL), and pyridoxal hydrochloride (2.03g, 10 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then β -naphthoyl chloride (2.12g, 12 mmoles) in acetone (20mL) was added and the mixture was stirred for two hours. The solid was filtered out and the solution was evaporated to dryness under vacuum. The residue was chromatographed according to Example 2.

[0118] The purified solid was analyzed according to Example 2, and the purity was confirmed according to Example 1.

[0119] Alternative to the above-described synthesis, the 3- β -naphthoate of pyridoxal is prepared by reacting the compound of Example 4 with 80% aqueous acetic acid at 60°C for 30 minutes, followed by diluting with water and extracting by ethyl acetate. The ethyl acetate layer is then washed with 5% aqueous sodium bicarbonate, dried with magnesium sulfate, and evaporated to dryness. The compound is also analyzed as described *supra*.

Example 6: Synthesis of 3-pivaloyl of the morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-pivaloyloxy)-6-methylfuro(3,4-c)pyridine)

[0120] Anhydrous powdered potassium carbonate (5g), acetone (100mL), and morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-c)pyridine)

(1.11g, 5 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then pivaloyl chloride (trimethylacetyl chloride) (720mg, 6 mmoles) in acetone (20mL) was added. The reaction mixture was stirred for two hours. The solid was then filtered out and the solution was evaporated to dryness under vacuum. The residue was chromatographed according to Example 2.

[0121] The purified solid was analyzed according to Example 2, and the purity was confirmed according to Example 1.

Example 7: Synthesis of 3-dimethylcarbamoyl of the morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-(dimethylcarbamoyloxy)-6-methylfuro(3,4-c)pyridine)

[0122] Anhydrous powdered potassium carbonate (5g), acetone (100mL), and morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-c)pyridine) (1.11g, 5 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then dimethylcarbamoyl chloride (642mg, 6 mmoles) in acetone (20mL) was added. The reaction mixture was stirred for two hours. The solid was then filtered out and the solution was evaporated to dryness under vacuum. The residue was chromatographed according to Example 2.

[0123] The purified solid was analyzed according to Example 2, and the purity was confirmed according to Example 1.

Example 8: Synthesis of 3-acetylsalicyloyl of the morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-acetylsalicyloxy)-6-methylfuro(3,4-c)pyridine)

[0124] Anhydrous powdered potassium carbonate (5g), acetone (100mL), and morpholine pyridoxal-4,5-aminal (1-morpholino-1,3-dihydro-7-hydroxy-6-methylfuro(3,4-c)pyridine) (1.11g, 5 mmoles) were mixed in a nitrogen-cooled, dry flask. The mixture was cooled to between 0 and 5°C and then acetylsalicyloyl chloride (1.09g, 6 mmoles) in acetone (20mL) was added. The reaction mixture was stirred for two hours. The solid was then filtered out and the solution was evaporated to dryness under vacuum. The residue was chromatographed according to Example 2.

[0125] The purified solid was analyzed according to Example 2, and the purity was confirmed according to Example 1.

Example 9: In Vitro - Ischemia Reperfusion in Isolated Rat Hearts and Measurement of Left Ventricular Developed Pressure (LVDP)

[0126] Male Sprague-Dawley rats weighing 250-300g are anaesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). The hearts are rapidly excised, cannulated to a Langendorff apparatus and perfused with Krebs-Henseleit-solution, gassed with a mixture of 95% O₂ and 5% CO₂, pH 7.4. The perfusate contained (in mM): 120 NaCl, 25 NaHCO₃, 11 glucose, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄ and 1.25 CaCl₂.

[0127] The hearts are electrically stimulated at a rate of 300 beats/min (Phipps and Bird Inc., Richmond, VA) and a water-filled latex balloon is inserted in the left ventricle and connected to a pressure transducer (Model 1050BP; BYOPAC SYSTEM INC., Goleta, California) for the left ventricular systolic measurements. The left ventricular end diastolic pressure (LVEDP) is adjusted at 10 mmHg at the beginning of the experiment. In some experiments the left ventricular pressures are differentiated to estimate the rate of ventricular contraction (+dP/dt) and rate of ventricular relaxation (-dP/dt) using the Acknowledge 3.03 software for Windows (BIOPAC SYSTEM INC.,) Goleta, California). All hearts are stabilized for a period of 30 min and then randomly distributed into nine different experimental groups (n= 5-8 per group). The experimental groups are defined as follows:

- 1) Control group (control hearts are further perfused for 90 minutes for a total of 130 min of continuous perfusion);
- 2) Ischemia reperfusion group (Ischemia reperfusion hearts are made globally ischemic by stopping the coronary flow completely for 30 min and then the hearts are reperused for 60 min);
- 3) P-5-P (15 uM) treated group;
- 4) captopril (100 uM) treated group;
- 5) verapamil (0.01 uM)) treated group;
- 6) propranolol (3mM) treated group;
- 7) PPADS (10 uM) treated group;

- 8) P-5-P + captopril treated group;
- 9) P-5-P + verapamil treated group;
- 10) P-5-P + propranolol treated group;
- 11) P-5-P + PPADS treated group.

[0128] Drug treatment is started 10 min before global ischemia followed by 30 min global ischemia and 60 min reperfusion. At the end of some experiments, the hearts are quickly freeze-clamped with a liquid nitrogen precooled Wollenberger tong. Rats are housed in clear cages in a temperature and humidity controlled room on a 12 hr light-dark cycle. Food and water are supplied *ad libitum*.

[0129] Hearts subject to 30 min of ischemia followed by 60 min of reperfusion showed slight recovery in the contractile function as represented by 29.5% recovery in LVDP (left ventricular developed pressure). As compared to the untreated group, treatment with P-5-P, captopril, or P-5-P and captopril showed better recoveries in LVDP by 78.2%, 61.4%, and 132% respectively (Table I).

Table I

Effect of Pyridoxal-5-phosphate (P-5-P, 15uM) and Captopril (100uM) on % recovery of left ventricular systolic pressure (LVDP).

Drugs	<u>LVDP</u>		<u>LVEDP</u>	<u>LVSP</u>	% recovery (LVDP)
	(B)	(A)	mmHg	mmHg	
Untreated	87±7	25±2.9	62±5.6	87±6.9	29.5±3.7
P5P	80±3.8	63±5	35±4.8	98±8.2	78.2 ± 3.3*
Captopril	78±10.9	47±8.6	54±6.7	101±14.6	61.4 ± 5.2*
P5P + Captopril	89±6.9	69±7.4	28±7.3	117±8.4	132 ± 7.5 [#]

(A) =After ischemia, (B) =Before ischemia.

[0130] Hearts subject to 30 min of ischemia followed by 60 min of reperfusion showed slight recovery in the contractile function as represented by 29.5% recovery in LVDP. As compared to the untreated group, treatment with P-5-P, verapamil, or P-5-P and verapamil showed better recoveries in LVDP by 78.2%, 43%, and 109% respectively (Table II).

Table II

Effect of Pyridoxal-5-phosphate (P-5-P,15uM) and Verapamil (0.01uM) on % recovery of left ventricular systolic pressure (LVDP).

Drugs	LVDP		LVEDP mmHg	LVSP mmHg	% recovery (LVDP)
	(B)	(A)			
Untreated	87±7	25±2.9	62±5.6	87±6.9	29.5±3.7
P5P	80±3.8	63±5	35±4.8	98±8.2	78.2 ± 3.3*
Verapamil	54±9.1	23±4.5	55±5.1	78±7.7	43 ± 6.6
P5P + Verapamil	78±10.5	85±11.7	34±7.3	119±8	109 ± 4.6 [#]

(A) =After ischemia, (B) =Before ischemia.

[0131] Hearts subject to 30 min of ischemia followed by 60 min of reperfusion showed slight recovery in the contractile function as represented by 29.5% recovery in LVDP. As compared to the untreated group, treatment with P-5-P, PPADS, or P-5-P and PPADS showed better recoveries in LVDP by 78.2%, 61%, and 128% respectively (Table III).

Table III

Effect of Pyridoxal-5-phosphate (P-5-P,15uM) and Pyridoxal phosphate 6-azophenyl-2'-4'disulfonic acid (PPADS 100uM) on % recovery of left ventricular systolic pressure (LVDP).

Drugs	LVDP		LVEDP mmHg	LVSP mmHg	% recovery (LVDP)
	(B)	(A)			
Untreated	87±7	25±2.9	62±5.6	87±6.9	29.5±3.7
P5P	80±3.8	63±5	35±4.8	98±8.2	78.2 ± 3.3*
PPADS	92±15.2	58±13.6	57±6.3	115±11.5	61 ± 4.8*
P5P + PPADS	82±15.8	105±22.8	34±3.1	139±21.6	128 ± 13.8 [#]

(A) =After ischemia, (B) =Before ischemia.

[0132] Hearts subject to 30 min of ischemia followed by 60 min of reperfusion showed slight recovery in the contractile function as represented by 29.5% recovery in LVDP. As compared to the untreated group, treatment with P-5-P, propranolol, or P-5-P and propranolol showed better recoveries in LVDP by 78.2%, 74%, and 120% respectively (Table IV).

Table IV

Effect of Pyridoxal-5-phosphate (P-5-P,15uM) and Propranolol (3uM) on % recovery of left ventricular systolic pressure (LVDP).

Drugs	<u>LVDP</u>		<u>LVEDP</u> <i>mmHg</i>	<u>LVSP</u> <i>mmHg</i>	% recovery (LVDP)
	<i>(B)</i>	<i>(A)</i>			
Untreated	87 \pm 7	25 \pm 2.9	62 \pm 5.6	87 \pm 6.9	29.5 \pm 3.7
P5P	80 \pm 3.8	63 \pm 5	35 \pm 4.8	98 \pm 8.2	78.2 \pm 3.3*
Propranolol	61 \pm 10.8	45 \pm 9.7	27 \pm 6.6	72 \pm 15.1	74 \pm 4.9*
P5P + Propranolol	67 \pm 12.6	75 \pm 10.4	40 \pm 4.2	115 \pm 8.3	120 \pm 15.5 [#]

(A) =After ischemia, (B) =Before ischemia

[0133] Tables I-IV demonstrate that P-5-P in addition to providing significant benefit in ischemia reperfusion injury when given alone also improves or adds to the benefits associated with other commonly used drugs when given in combination with these drugs.

[0134] In addition to captopril, other angiotensin converting enzyme inhibitors, such as, for example, enalapril or imidapril, can similarly be administered in place of captopril. In addition to verapamil, other known calcium channel blockers, such as, for example, nifedipine or diltiazem, can similarly be administered in place of verapamil. In addition to propranolol, other β -adrenergic receptor antagonists such as, for example, atenolol, timolol, and metoprolol can similarly be administered in place of propranolol. Additionally, angiotensin II receptor antagonists such as, for example, losartan and valsartan can be used in the above example.

Example 10: In Vivo - Coronary Artery Ligation

[0135] Myocardial infarction is produced in male Sprague-Dawley rats (200-250 g) by occlusion of the left coronary artery as described in Sethi et al., J. Cardiac Failure, 1(5) (1995) and Sethi et al., Am. J. Physiol., 272 (1997).

[0136] Rats are anesthetized with 1-5% isoflurane in 100% O₂ (2L flow rate). The skin is incised along the left sterna border and the 4th rib is cut proximal to the sternum and a retractor inserted. The pericardial sac is opened and the heart externalized. The left anterior descending

coronary artery is ligated approximately 2 mm from its origin on the aorta using a 6-0 silk suture. The heart is then repositioned in the chest and the incision closed via purse-string sutures.

[0137] Sham operated rats undergo identical treatment except that the artery is not ligated. Mortality due to surgery is less than 1%. Unless indicated in the text, the experimental animals showing infarct size >30% of the left ventricle are used in this study. All animals are allowed to recover, allowed to receive food and water ad libitum, and are maintained for a period of 21 days for Electrocardiogram (ECG), hemodynamic, and histological assessment.

[0138] Occlusion of the coronary artery in rats has been shown to produce myocardial cell damage which results in scar formation in the left ventricle and heart dysfunction. While the complete healing of the scar occurs within 3 weeks of the coronary occlusion, mild, moderate and severe stages of congestive heart failure have been reported to occur at 4, 8 and 16 weeks after ligation. Accordingly, the contractile dysfunction seen at 3 weeks after the coronary occlusion in rats is due to acute ischemic changes.

[0139] The rats are housed in clear cages in a temperature and humidity controlled room, on a 12 hour light-dark cycle. Food and water are supplied ad libitum. After surgery, rats are randomly assigned to treatment or non-treatment in both sham and experimental groups. Randomization of animals was performed and treatment begins 1 hour after coronary occlusion and continues for 21 days. The total duration of experiments in each case is 21 days. The groups are as follows:

- 1) sham operated;
- 2) coronary artery ligated (treatment with equal volumes of saline);
- 3) coronary artery ligated (treated with 10 mg/kg P-5-P);
- 4) coronary artery ligated (treated with 100 mg/kg captopril);
- 5) coronary artery ligated (treated with 50 mg/kg propranolol);
- 6) coronary artery ligated (treated with 100 mg/kg aspirin);
- 7) coronary artery ligated (treated with 25 mg/kg verapamil) ;
- 8) coronary artery ligated (treated with 10 mg/kg P-5-P + 100 mg/kg captopril);
- 9) coronary artery ligated (treated with 10 mg/kg P-5-P + 50 mg/kg propranolol);

- 10) coronary artery ligated (treated with 10 mg/kg P-5-P + 100 mg/kg aspirin);
- 11) coronary artery ligated (treated with 10 mg/kg P-5-P + 25 mg/kg verapamil).

[0140] P-5-P (10 mg/kg), captopril (100 mg/kg), propranolol (50 mg/kg), verapamil (25 mg/kg) and aspirin (100 mg/kg) were administered once daily by gastric tube.

[0141] Acute myocardial infarction resulted in a total mortality of 35% % in the untreated group of rats in 21 days. The highest mortality occurred within the first 2 days following occlusion. As compared to the untreated group, treatment with P-5-P, aspirin, or P-5-P and aspirin showed lower mortality rates of 15%, 25%, 15%, respectively (Figure 1).

[0142] Acute myocardial infarction resulted in a total mortality of 35% % in the untreated group of rats in 21 days. The highest mortality occurred within the first 2 days following occlusion. As compared to the untreated group, treatment with P-5-P, captopril, or P-5-P and captopril showed lower mortality rates of 10%, 15%, 20%, respectively (Figure 2).

[0143] Acute myocardial infarction resulted in a total mortality of 35% % in the untreated group of rats in 21 days. The highest mortality occurred within the first 2 days following occlusion. As compared to the untreated group, treatment with P-5-P, propranolol, or P-5-P and propranolol showed lower mortality rates of 15%, 20%, 20%, respectively (Figure 3).

[0144] Acute myocardial infarction resulted in a total mortality of 35% % in the untreated group of rats in 21 days. The highest mortality occurred within the first 2 days following occlusion. As compared to the untreated group, treatment with P-5-P, verapamil, or P-5-P and verapamil showed lower mortality rates of 15%, 25%, 10%, respectively (Figure 4).

[0145] In addition to captopril, other angiotensin converting enzyme inhibitors, such as, for example, enalapril or imidapril, can similarly be administered in place of captopril. In addition to verapamil, other known calcium channel blockers, such as, for example, nifedipine or diltiazem, can similarly be administered in place of verapamil. In addition to propranolol, other β -adrenergic receptor antagonists such as, for example, atenolol, timolol, and metoprolol can

similarly be administered in place of propranolol. In addition to aspirin, other antithrombolytic agents such as, for example, antiplatelet agents and heparin can similarly be administered in place of aspirin. Additionally, angiotensin II receptor antagonists such as, for example, losartan and valsartan can be used in the above example.

[0146] These animals are used in Examples 11 and 12 below. For EKG studies, these animals are used as their controls before surgery, so that before surgery is done on these animals EKG traces are taken which are then used as controls for the same animals after surgery.

Example 11: In Vivo - Hemodynamic Changes

[0147] The animals are prepared and grouped as described in Example 10 and were anesthetized with a solution of ketamine/xylazine which was injected. To maintain adequate ventilation, the trachea was intubated; the right carotid artery was exposed for introducing a microtip pressure transducer (model SPR-249, Millar, Houston, TX) into the left ventricle. The catheter was secured with a silk ligature around the artery, and various hemodynamic parameters such as left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), rate of contraction (+dp/dt), rate of relaxation (-dp/dt) were recorded and calculated on a computer system using a Acknowledge 3.1 software.

[0148] Once the hemodynamic parameters were measured the animals were sacrificed and hearts removed for measurement of heart weight, right ventricular weight, left ventricular weight and scar weight. Because complete healing of the scar in rats after coronary occlusion requires approximately 3 weeks, scar weight were measured only at 21 days.

[0149] Figures 5-8 demonstrate that the occlusion of coronary artery in rats for 21 days produces a significant scar evident by scar weight. Furthermore, Figures 5-8 demonstrate that P-5-P has a significant beneficial effect on scar weight in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0150] Figures 9-12 demonstrate that P-5-P has a significant beneficial effect on rate of contraction (+dP/dt) in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0151] Figures 13-16 demonstrate that P-5-P has a significant beneficial effect on rate of relaxation (+dP/dt) in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0152] Figures 17-20 demonstrate that P-5-P has a significant beneficial effect on rate of left ventricular end diastolic pressure (LVEDP) in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0153] Figures 21-24 demonstrate that P-5-P has a significant beneficial effect on whole heart weight in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0154] Figures 25-28 demonstrate that P-5-P has a significant beneficial effect on right ventricular weight in groups where P-5-P treatment is either given alone or in combination with verapamil, aspirin, captopril, or propranolol, respectively.

[0155] In addition to captopril, other angiotensin converting enzyme inhibitors, such as, for example, enalapril or imidapril, can similarly be administered in place of captopril. In addition to verapamil, other known calcium channel blockers, such as, for example, nifedipine or diltiazem, can similarly be administered in place of verapamil. In addition to propranolol, other β -adrenergic receptor antagonists such as, for example, atenolol, timolol, and metoprolol can similarly be administered in place of propranolol. In addition to aspirin, other antithrombolytic agents such as, for example, antiplatelet agents and heparin can similarly be administered in place of aspirin. Additionally, angiotensin II receptor antagonists such as, for example, losartan and valsartan can be used in the above example.

Example 12: In Vivo - Hypertension

[0156] It has been well demonstrated by various investigators that feeding 8-10% sucrose in water induces hypertension in rats. Zein et al., Am. Coll. Nutr., 17 (1), 36-37, 1998; Hulman et al., Pediatr. Res., 36:95-101; Reaven et al., Am. J. Hypertens; 1991:610-614. In applying this model, the concurrent administration of pyridoxal-5'-phosphate and captopril or verapamil significantly decreases the sucrose-induced increase in systolic blood pressure (SBP).

[0157] The blood pressure is monitored using the tail cuff method. The SBP is detected on an amplifier and the Acknowledge™ computer software program is used to determine the calculations.

[0158] The effect of concurrent administration of pyridoxal-5'-phosphate and captopril or verapamil on systolic blood pressure (marker of hypertension) in 10% sucrose induced hypertension in rats is determined.

[0159] Figures 29A and 9B demonstrate that P-5-P has a significant beneficial effect on systolic blood pressure in groups where P-5-P treatment is either given alone or in combination with verapamil or captopril 1 week prior to inducing hypertension in rats with a sucrose diet.

[0160] Figures 29A and 29B demonstrate that P-5-P has a significant beneficial effect on systolic blood pressure in groups where P-5-P treatment is either given alone or in combination with verapamil or captopril 1 week prior to inducing hypertension in rats with a sucrose diet.

[0161] Figures 30A and 30B demonstrate that P-5-P has a significant beneficial effect on systolic blood pressure in groups where P-5-P treatment is either given alone or in combination with verapamil or captopril the same day as inducing hypertension in rats with a sucrose diet.

[0162] Figures 31A and 31B demonstrate that P-5-P has a significant beneficial effect on systolic blood pressure in groups where P-5-P treatment is either given alone or in combination with verapamil or captopril two weeks after inducing hypertension in rats with a sucrose diet.

[0163] In addition to captopril, other angiotensin converting enzyme inhibitors, such as, for example, enalapril or imidapril, can similarly be administered in place of captopril. In addition to verapamil, other known calcium channel blockers, such as, for example, nifedipine or diltiazem, can similarly be administered in place of verapamil. In addition to propranolol, other β -adrenergic receptor antagonists such as, for example, atenolol, timolol, and metoprolol can similarly be administered in place of propranolol. Additionally, angiotensin II receptor antagonists such as, for example, losartan and valsartan can be used in the above example.

[0164] It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds.

[0165] Although embodiments of the invention have been described above, it is not limited thereto, and it will be apparent to persons skilled in the art that numerous modifications and variations form part of the present invention insofar as they do not depart from the spirit, nature, and scope of the claimed and described invention.

[0166] All references, applications, and patents cited herein are incorporated by reference.